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Maillard Reaction of D-Glucose: Identification of a Colored Product with Hydroxypyrrole and Hydroxypyrrolinone Rings Connected by a Methine Group

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Investigation of the colored products formed by the reaction of D-glucose with butylammonium acetate has been extended. The previously unknown 1-*N*-butyl-4-hydroxy-5-methyl-2-(*N*-butyl-3-hydroxy-5-(2-hydroxyethyl)pyrrolyl-2-methylidene)-2*H*-pyrrolin-3-one (**2a**) was isolated from the reaction mixture and identified after acylation by spectroscopic data. Butylaminammonium acetate was used as a model compound representing the lysine side chains of proteins.

KEYWORDS: Maillard reaction; color formation; D-glucose

INTRODUCTION

The Maillard reaction of reducing sugars with amino acids or proteins generally leads to a great variety of products. These processes are of great importance in many heated foods. So far, several low molecular weight compounds absorbing in the ultraviolet region have been characterized, but only a few colored compounds could be isolated in pure form from hexose reaction mixtures. On the other hand, color reactions of pentoses have been investigated in more detail, but obviously the degradation sequences of hexoses and pentoses are essentially different (*1*, *2*). When reducing sugars are heated with amino acids, proteins, or simple primary amines, many colored substances are formed but in low concentration compared to other products. As a consequence, isolation and purification of individual colored compounds proved to be difficult. Here, we report on the structural identification of a previously unknown compound, which is formed in a reaction mixture of D-glucose and butylammonium acetate in aqueous solution. Butylammonium acetate is a model compound representing the lysine side chains of proteins. Several investigations lead to the conclusion that Maillard reactions of simple primary amines, amino acids, α-*N*-acetyllysine, or proteins lead to the same heterocyclic structures which differ only in the nitrogen substituents.

MATERIALS AND METHODS

Apparatus. ^{1}H nuclear magnetic resonance (NMR) (500 MHz), ^{13}C NMR (125 MHz), H,H-COSY (correlated spectroscopy), HMQC (heteronuclear multiquantum coherence), and HMBC (heteronuclear multibond correlation) spectra were recorded with a JEOL Eclipse+500 spectrometer. Chemical shifts are reported in parts per million relative to $(CH_3)_4Si$ as internal standard. Mass spectrometric analyses were obtained with an electrospray instrument Sciex API 2000, and high-resolution electrospray mass spectra (HR-ESI-MS) were obtained with a Finnigan MAT 95Q spectrometer. Thin-layer chromatography was performed using 20 cm × 20 cm glass plates coated with a 0.5 mm thickness of silica gel (Merck, Darmstadt, Germany). Column chromatography was performed on silica gel 230−400 mesh, 60 A (Merck) by applying slight pressure. Analytical high-performance liquid chromatography (HPLC) was performed with a Merck Hitachi L-6000 pump and an L-4000 UV detector. The eluent was methanol/water (1:1) with a flow rate of 0.4 mL/min, and the column was a Nucleosil 100-5 C_{18} (Machery and Nagel, Düren, Germany). The detection wavelength was 450 nm.

Isolation of 1-*N*-Butyl-4-acetoxy-5-methyl-2-(*N*-butyl-3-hydroxy-5-(2-acetoxyethyl)pyrrolyl-2-methylidene)-2*H*-pyrrolin-3-one (2b). A mixture of D-glucose (12.6 g, 0.07 mol), *n*-butylamine (10 mL, 0.1 mol), and acetic acid (7 mL, 0.12 mol) in 50 mL of water was heated for 40 min under reflux. After it was cooled, the solution was extracted with 250 mL of ethyl acetate. The organic layer was dried with sodium sulfate and concentrated under reduced pressure. The mixture was separated by column chromatography on silica gel (12 cm × 5.5 cm i.d., eluent ethyl acetate/methanol 9:1). Three successive fractions (A, B, and C) of ∼30 mL were collected and checked by HPLC. Fractions B and C contain substances **1a** and **2a**, respectively, as the main components. Fraction C was evaporated under reduced pressure, acetylated with acetic anhydride in pyridine for 1 h at room temperature, and stored at about −4 °C for 12 h. After the addition of ice water and ethyl acetate, the organic layer was washed five times with water, dried over sodium sulfate, and evaporated under reduced pressure. The product was purified by thin-layer chromatography on silica gel plates using ethyl acetate as eluent. An intense yellow zone (R_f 0.46, the lower of two yellow zones) was eluted and again purified by thin-layer chromatography with acetonitrile/trichloromethane (1:1) as eluent. **2b** was obtained as a yellow-orange oil (∼0.1% isolated yield): HR-ESI-(+)-MS, calcd for $C_{24}H_{35}N_2O_6$, 447.2495 (M + 1), found, 447.2452; ^{1}H NMR ($CDCl_3$; arbitrary numbering of carbon atoms refers to **Figure 2**) δ 0.96 (t, 3H, $^3J_{10,11}$ = 7.0 Hz, H-11), 0.98 (t, 3H, $^3J_{19,20}$ = 7.0 Hz, H-20), 1.38 (m, 4H, H-10, H-19), 1.64 (m, 4H, H-9, H-18), 2.07, 2.33 (2s, 6H, H-22, H-24), 2.19 (s, 3H, H-16), 2.94 (t, 2H, $^3J_{6,7}$ = 7.0 Hz, H-6), 3.83 (m, 4H, H-8, H-17), 4.33 (t, 2H, $^3J_{6,7}$ = 7.0 Hz, H-7), 5.73

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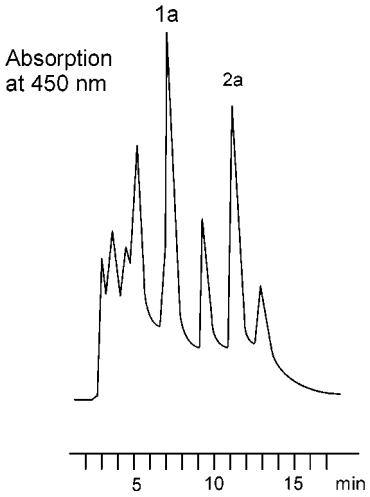

Figure 1. HPLC control of raw extract containing **1a** and **2a**.

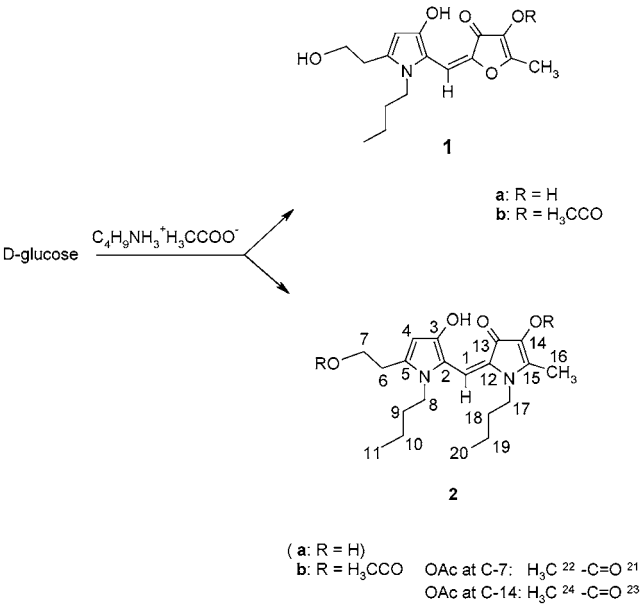

Figure 2. Formation of colored compounds from D-glucose and butylammonium acetate in aqueous solution. Numbering of the carbon atoms in structure **2** is arbitrary.

(s, 1H, H-4), 6.72 (s, 1H, H-1), 12.40 (s, 1H, HO-3); ^{13}C,DEPT135, HMQC, HMBC, 10.43 (C-16), 13.79 (C-20), 13.80 (C-11), 20.25 (C-19), 20.29 (C-10), 20.51 (C-24), 20.91 (C-22), 26.99 (C-6), 32.66 (C-18), 32.78 (C-9), 43.26 (C-17), 43.41 (C-8), 61.83 (C-7), 101.43 (C-4), 110.88 (C-1), 119.88 (C-2), 126.12 (C-14), 128.58 (C-12), 141.44 (C-15), 148.23 (C-5), 160.50 (C-3), 168.64 (C-13), 169.13 (C-21), 170.90 (C-23); UV−vis (CH_3OH), λ_{max} (log ϵ) 454 nm (4.11).

RESULTS AND DISCUSSION

When D-glucose and butylammonium acetate were heated in aqueous solution, an orange color developed within a few minutes, which turned dark brown during prolonged heating. Several colored products could be extracted with ethyl acetate from the reaction mixture. A HPLC chromatogram registered at 450 nm is shown in **Figure 1**. The extract was separated into crude fractions by chromatography on a silica gel column with ethyl acetate/methanol (9:1) as solvent. Two main colored products, **1a** and **2a**, could be separated with fractions B and C, respectively. Recently compound **1a** (**Figure 2**) has been isolated and identified as a substance with hydroxypyrrole and furanone rings connected by a methine group (*3*). The extended

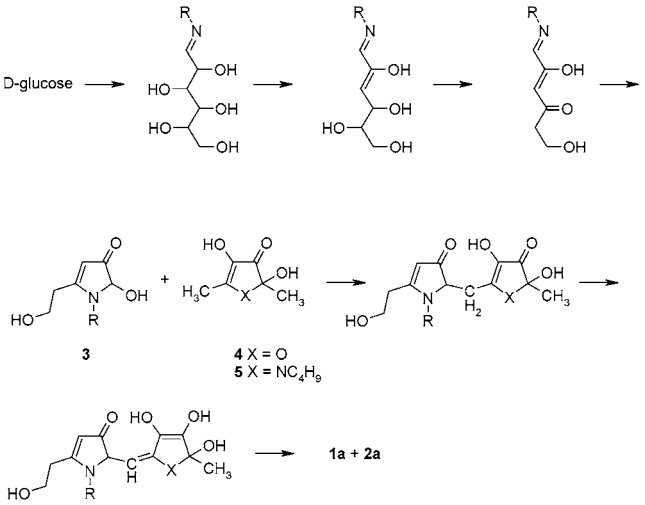

Figure 3. Proposed reaction mechanism leading from D-glucose to **1a** and **2a**.

conjugated system explains the orange color with an absorption maximum at 453 nm (in methanol). All of our attempts to obtain the main compound of fraction C as a pure substance by several chromatographic procedures failed. The substance slowly degraded under ambient conditions. A stable derivative was obtained by acylation with acetic anhydride in pyridine. The structure of the purified compound **2b** (**Figure 2**) was derived from the MS spectra and NMR spectroscopic data. There is a striking similarity between the NMR data of the previously identified compound **1b** (*1*) and those of the new product **2b**. The only differences of **1b** are given by the oxygen substitution in the furanone ring by butylamine and a second *O*-acetyl group at C-7. Therefore, the chemical shifts in proton and carbon spectra differ only at C-7, C-12, C-14, and C-15, whereas the other differences are negligible. The couplings of carbon atoms and protons via two and three bonds in HMBC data prove the nearly identical structure, for example, C-2, C-3, and C13 with H-1; C-5 with protons H-4, H-6, H-7, H-8; C-2 with protons H-1, H-4, and H-9 or C-15 with protons H-16 and H-17. The λ_{max} in the UV−vis shows a 3 nm difference (**1b**, λ_{max} = 457 nm) with nearly the same π-electron system.

So far, the reaction mechanism explaining the formation of compounds **1a** and **2a** has not rigorously been established. As proposed for the formation of **1a**, the (hypothetical) D-glucose degradation product **3** may react with acetylformoin **4** to afford the condensation product **1a** (**Figure 3**). By analogy, compound **3** may react with the pyrrolinone **5** to give the new compound **2a**. Acetylformoin **4** and the pyrrolinone **5** are well-known sugar degradation or Maillard reaction products, respectively (*4*). With regard to these aspects the investigations of Hofmann (*5*) are of special interest. After the addition of furanaldahyde to Maillard reaction mixtures, he was able to isolate condensation products of furanaldehydes with acetyformoin **4** and the hydroxypyrrolinone **5**. On the basis of these results it was concluded that the sugar degradation products **4** and **5** are involved in the formation of colored Maillard reaction products. Our results substantiate this proposal. It must be emphasized that the Maillard reaction is highly dependent on the reaction conditions. In alcoholic solution or in mixtures of low water content a yellow compound of structure **6** (**Figure 4**) is formed as a colored product (*6*). The compounds described in this and the preceding papers are sufficiently lipophilic to be extractable from an aqueous solution by organic solvents. On the other hand, many Maillard products are more hydrophilic and remain in the aqueous phase. This group of compounds is at present under

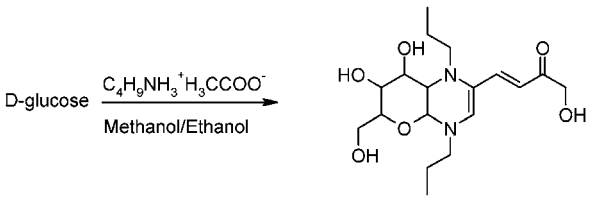

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Figure 4. Structure of a colored compound from D-glucose and propylammonium acetate in alcoholic solution.

investigation. After prolonged heating, dark brown products dominate and are difficult to separate into pure compounds.

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